INDUCTION OF INFLAMMATORY CYTOKINE EXPRESSION
IN EPITHELIAL CELLS AND CULTURED
CHOLANGIOCARCINOMA CELLS IN RESPONSE TO
OPISTHORCHIS VIVERRINI ANTIGEN STIMULATION

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The induction of inflammatory cytokine expression in epithelial cells and cultured cholangiocarcinoma cells in response to *Opisthorchis viverrini* antigen stimulation

**Abstract**

Cholangiocarcinoma (CCA) is a significant problem for Thailand, especially in the eastern and northeastern parts of the country. Recent improvements in diagnosis and treatment have not yet reached an acceptable level. The infection with *Opisthorchis viverrini* is a significant factor in the development of CCA. In addition, the infection with chronic inflammation is considered a risk factor for the development of this type of cancer. However, the mechanisms of *Opisthorchis viverrini* infection that lead to the development of CCA remain unclear.

The purpose of this study is to demonstrate the induction of inflammatory cytokine expression in epithelial cells and cultured cholangiocarcinoma cells in response to *Opisthorchis viverrini* antigen stimulation. The techniques used were Reverse transcription-polymerase chain reaction (RT-PCR) to test the expression of cytokine mRNA and Enzyme-linked immunosorbent assay (ELISA) to test the expression of cytokine protein in the stimulated cells.

From this study, we found that even without any stimulation, some cholangiocarcinoma cells showed expression of cytokine mRNA, which included pro-inflammatory cytokines (IL-1α, IL-1β, IL-6, IL-8) and anti-inflammatory (TGF-α, TGF-β1) cytokines. After stimulation with *Opisthorchis viverrini* antigen, there was a significant increase in the expression of cytokine protein and mRNA in the studied cells. The response to the same antigenic type varied depending on the type of cell tested.

**Keywords:** Cholangiocarcinoma, *Opisthorchis viverrini*, Cytokine, RT-PCR, ELISA.
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ABSTRACT

Cholangiocarcinoma (CCA), a bile-duct epithelial cancer, is a serious public health problem, particularly in the northeastern part of Thailand. There are currently no effective means of diagnosis or treatment for this disease. It is known that infection with liver fluke, *Opisthorchis viverrini*, is associated with an increased risk of developing CCA. Although *O. viverrini* infection is a known causal factor for CCA development, the mechanism by which these worms involve in carcinogenesis is unclear. Furthermore, chronic infection and inflammation are also risk factors for the development of CCA. However, the link between inflammation and carcinogenesis is still obscure. The objective of this study was to evaluate the inflammatory cytokine production that occurs in response to *O. viverrini* stimulation. We investigated the *in vitro* response of cultured human epithelial and CCA cell lines to *O. viverrini* antigen stimulation. Reverse transcription-polymerase chain reaction (RT-PCR) and enzyme-linked immunosorbent assay (ELISA) were applied to evaluate the mRNA expression and protein level of cytokine in the spent culture fluid, respectively.

The results showed that human CCA cell lines constitutively expressed several pro-inflammatory (IL-1α, IL-1β, IL-6, IL-8) and anti-inflammatory (TGF-α, TGF-β1) cytokines. Subsequently, human CCA cells were stimulated with *O. viverrini* antigens, there were only small changes in the cytokine mRNA expression. By contrast, a significant increase of both pro-inflammatory (IL-1β, IL-6, IL-8) and also anti-inflammatory (TGF-β1) cytokine proteins was observed. Similar results were observed when the lung epithelial (A549) cell line was subsequently used for comparison. By contrast, a significant decrease in production of IL-8 by the colon epithelial (Caco-2) cell line was observed after stimulated with *O. viverrini* antigens. In conclusion, although different human epithelial cell lines could be stimulated with the same *O. viverrini* antigen preparations, the amount and overall pattern of the inflammatory cytokine production in response to parasite stimulation varied from one cell line to another.

KEY WORDS: CHOLANGIOCARCINOMA/ *Opisthorchis viverrini*/ INFLAMMATORY CYTOKINE/ ELISA/ RT-PCR

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